Bc. Petra Tocháčková

Antimicrobial peptides in infections with grammnegative bacteria

Diploma thesis - abstract
Charles University in Prague, Faculty of Pharmacy in Hradec Králové
Pharmacy

Background: Aim of this thesis was a development of methods for detection of gene activation of antimicrobial peptides (beta defensins 1 and 2 – pBD1 and pBD2) in ileum during infection of gnotobiotic piglets with gramnegative bacteria. *Salmonella enterica* serovar Typhimurium strain LT2 and its R mutants (*rfaG*- and *rfaL*-) were selected as representants of gramnegative bacteria of different virulence.

Methods: 1) Suggestion of systems for detection of pBD1 and pBD2 (software), 2) Purification of total RNA (commercial columns), quantification (spectrophotometry), and estimation of its purity (ratio A_{280}/A_{260}), 3) Reverse transcription (mixture of random n-mers and oligo $d(T)_m$), 4) Quantification of cDNA (real time polymerase chain reaction – LNA probes), and 5) Normalization of gene transcription for pBD1 and pBD2 against beta actin, cyclophilin A or to both together.

Results: We used a method reducing risk of contamination by genomic DNA and obtaining of false positive results for purification of total RNA. We suggested detection systems for estimation of pBD1 and pBD1 with efficiency approx. 100% (comparable with efficiencies of used housekeeping genes). The transctiption of pBD1 was not influenced by absence of *Salmonella* (germ-free piglets) or its virulence. The differences in activation of gene transcription of pBD2 were significant in beta actin used as housekeeping gene, but not in case of cyclophilin A.

Conclusions: An influence of virulence on expression of the porcine beta defensins in the gnotobiotic piglets infected/associated with gramnegative bacteria *Salmonella enterica* serovar Typhimurium was not unequivocally documented. However we pointed a possibility of this pathway to look for other more suitable microorganisms and their vaccine strains. A support of inner defense individual mechanisms would be one of the simplest attitudes how to protect against life-threatening infection. Using of vaccine strains stimulating production of endogenous antimicrobial peptides would be interesting either in medicine or in veterinary practice.

We confirmed a suitability of normalization of transcripts for pBD1 and pBD2 to different housekeeping genes and to their combinations.